

Prenatal Exposure To An Extremely Low Frequency-Low Intensity Rotating Magnetic Field And Increases In Thyroid And Testicle Weight In Rats

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Extremely low frequency (ELF) electromagnetic fields and waves (.1-40 Hz) which occur daily in the environment, are associated with lightning discharges, atmospheric labiality, solar eruptions, and geomagnetic micropulsations. In 3 experiments, adult rats that had been exposed to a .5-3 or 3-15 gauss ELF (.5 Hz) rotating magnetic field (RMF) during various periods of gestation, had significantly heavier thyroid and testicle weights than controls. The difference in these measures was a function of the field intensity and duration of exposure. Rats exposed prenatally to a sham-RMF (no magnets) did not differ from controls in the above measures. No significant differences were found between the thymus weights, adrenal weights, blood sugar, or circulating blood eosinophil levels of RMF-exposed and control rats. The implications of the thyroid and testicle changes are discussed in terms of the physiological and physicochemical effects of ELF electromagnetic phenomena.

Extremely low frequency (ELF) electromagnetic fields (.1 to 40 Hz, Ludwig & Ranscht-Froemsdorff, 1966) and very low frequency (VLF) carrier waves modulated with ELF pulses are associated with tropospheric labiality ranging from lightning discharges as distant as the Amazon Valley and central Africa (Holzer & Deal, 1965) to atmospheric changes as close as a few kilometers from the measurement point (König, 1962). Different frequencies within the ELF range are associated with different types of weather. Electromagnetic (EM) waves of 2-5 Hz are "top-waved" and appear before thunderstorms (König & Ankermüller, 1960) and during rain or heavy deep lying

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clouds (König, 1962). Signals of 9-10 Hz (Schumann resonance), which show sine-like oscillations, are apparently produced by strong lightning and show diurnal changes in intensity. Sine-like ELF signals between .5-2 Hz have also been measured, but their origin is unclear. During stable weather conditions, ELF pulse frequencies of 1-3 Hz superimposed upon a 10 kHz carrier wave have been measured, while during unstable weather conditions (e.g., close passage of a cold and warm front), a marked increase in the incidence of pulse frequencies of 30-100 Hz, superimposed upon a 10-100 kHz carrier have been measured (Lotmar, Ranscht-Froemsdorff, & Weise, 1969a). ELF-EM waves also show a significant increase 29 days following 200 MHz bursts associated with solar eruptions (Aarons & Henissart, 1953). Even the geomagnetic field has an ELF component (Graf, Cole, Weathers, Sims, & Johnson, 1967; Campbell, 1967). Local geophysical-geographical variables such as underground water level, mineral content of water, and altitude, affect ELF-EM wave and VLF carrier distributions (Ranscht-Froemsdorff, 1968; Ranscht-Froemsdorff & Weise, 1969; Ludwig, Mecke, & Seelewind, 1968).

The intensities of geomagnetic pulsations average less than 1 gamma (the intensity of the main "static" dipole field of the earth is about 5×10^4 gamma; .5 gauss) with some cavity resonances typically measuring .2 mV/m (Campbell, 1967). The intensities of the electrical component of the ELF waves range from >100 mV/m to <1 mV/m, while the magnetic component is about 10^{-5} A/m (<1 gamma). The energy available, for instance, to a synaptic cleft from these fields has been calculated to be 1.5×10^{-14} ergs (Ludwig & Ranscht-Froemsdorff, 1966). This is well within the energy change of 0.5×10^{-14} ergs which has been suggested to occur in a synaptic cleft during miniature excitatory postsynaptic potentials (Eccles, 1964).

Despite their low intensities, ELF-EM phenomena are of potential biological and behavioral significance for at least three reasons: (1) their intensities do not appreciably attenuate with distance (they can travel thousands of kilometers); (2) they penetrate buildings (unlike radio frequencies, ELF-EM signals cannot be completely shielded out by Faraday cages or steel-concrete buildings; Ranscht-Froemsdorff, 1969); and (3) they occur in the same frequency band as electrical phenomena associated with the brain (slow potentials, dc potentials, EEG), viscera (heart rate, respiration rate) and mechanical microvibrations of the body (Rohracher, 1955).

Extremely low frequency electromagnetic phenomena have been shown to be associated with a variety of behavioral changes (Persinger, Persinger, Ossenkopp, & Glavin, 1972; Ludwig *et al.*, 1968; Friedman, Becker, & Bachman, 1967; Reiter, 1964; König, 1962), and have been implicated as possible "zeitgebers" for circadian rhythms in man (Wever, 1967, 1968). Recently, Lotmar and Ranscht-Froemsdorff (1968) noted a decrease in respiration rate of rabbit skin during periods of unstable meteorological conditions, e.g., frontal passages, and an increase in respiration rate during periods of stable weather conditions. Assuming that the decrement in respiration rate during labile conditions was associated with an increased incidence of ELF-fields, Lotmar, Ranscht-Froemsdorff, and Weise (1969, a,b) exposed mouse liver

tissue in a Warburg apparatus to ELF-pulse frequencies of 1-3 Hz on a 10 kHz (<10 mV/m) carrier band (simulating anticyclonic stable weather conditions) and 30-100 Hz pulse frequencies on a 10-100 kHz (>100 mV/m) carrier band (simulating cyclonic-unstable conditions). A 42% decrease in respiration rate of the tissue was noted within 30 min of initial exposure to the 30-100 Hz pulse frequencies whereas no significant changes were noted with the 1-3 Hz pulses. Altmann (1969) found that a 1.75 Hz field (no carrier wave) of 40 V/m also produced a decrease in oxygen uptake in several species. Piccardi (1962) reported that VLF (10 kHz) frequencies (he did not mention possible ELF pulses) specifically affect the clotting-precipitation rate of colloids, e.g., blood in water solution. However, the above data in conjunction with statements by Ludwig and Ranscht-Froemsdorff (1966) suggest that the ELF pulses or fields and not the VLF carrier waves are the effective biotrophic weather factors.

Rats exposed during their entire prenatal development to an ELF (.5 Hz) rotating magnetic field (RMF) show decreased ambulatory behavior and increased defecation in an open field situation (Ossenkopp, 1972; Persinger, 1969), fewer lever presses in a Sidman avoidance situation (Persinger & Foster, 1970), and greater suppression of response rate in a conditioned suppression paradigm (Persinger & Pear, 1972). These behavioral data in combination with the reported decrements of respiration rate suggest that prenatal exposure to ELF fields might be associated with later measurable changes in tissue (by altering the developing tissue) of adult rats. In the present study we examined this possibility by measuring thyroid, testicle, and adrenal weights, as well as circulating blood eosinophil counts and blood sugar levels. Since ELF-exposed animals have a higher mortality rate than controls between 40-60 days of age (M.A. Persinger, K.P. Ossenkopp, and T. Koltek, unpublished data) and the thymus is associated with immunological reactions, this tissue was also selected for measurement.

Method

Subjects

Twenty 3 to 5 month old primiparous Holtzman strain albino rats (*Ratus norvegicus*), obtained from Holtzman Farms, Madison, Wisconsin, were used as breeders. On days that spermatozoa were found in the vaginal smears, 8 females were exposed at various periods to a rotating magnetic field, whereas the remaining 12 pregnant females were used as controls. Two control litters spent their gestation in the apparatus after the magnets had been removed.

In three experiments 20 male and 12 female rats from litters exposed prenatally at different periods to a .5 Hz, .3-15 gauss magnetic field, and 12 male and 10 female rats from 8 control litters were randomly selected as subjects. In experiment I rats were exposed during their entire gestation to a 3-15 gauss field, while those in experiment II were exposed only during the last 3 days of gestation. Rats in

experiment III were exposed during their entire gestation to a .5-3 gauss field. In a fourth experiment, 4 male rats from the 2 litters that had been exposed during their gestation to the apparatus after the magnets had been removed (sham-field) and 4 male rats from 2 litters exposed to usual control conditions were used as subjects. The males and females had been tested on conditioned suppression and delayed conditioned approach procedures (respectively) that had been terminated 2 weeks before the experiments.

Apparatus and Procedure

A description of the rotating magnetic field (RMF) and exposure procedure used in this study is reported earlier (Persinger & Pear, 1972, Persinger, 1969). Briefly, the RMF was created by two horseshoe magnets rotating (via an electric motor) at 30 rpm in opposite directions about their major axes that were aligned in a N-S direction in Winnipeg, Canada. The mothers of the subjects used in the present experiments were placed in rubber tile cages on the day spermatozoa were found in the vaginal smears. In experiments I and III, cages were then placed in either the control or RMF conditions. In experiment II one cage was placed in the running apparatus with the magnets off until Day 19 (Day 1 being the day spermatozoa were found in the vaginal smear) when the magnets were added. In experiment IV one cage was placed in the running apparatus with the magnets removed (sham-field) during the entire experimental period while the other cage was placed in the usual control conditions. During exposure lighting was continuous and furnished by a fluorescent lamp (20 W) 45 cm above each cage. Food and water were freely available. Noise (59 dB in the RMF area and 57 dB in the control area), temperature, and humidity were recorded but did not differ significantly between areas.

The pups and mothers were removed from the experimental conditions within 6-8 hrs after birth and placed in the department's colony room (light-dark cycle 12:12). After weaning at 21 days of age, 2 animals of the same sex and conditions were placed in 17 X 17 X 25-cm steel cages where food and water were freely available. From 70-91 days of age, the RMF-exposed and control females were maintained on 23 hr water deprivation and tested in a delayed conditioned approach paradigm as described by Halasz (1968) and Halasz, Hughes, Humpherys, & Persinger (1970). After testing water was again freely available. Control and RMF-exposed males were maintained on 80% free feeding weight (with compensation for growth) from 70 days of age until 130 days of age in experiment I and 105 days in experiments II, III and IV, and tested for 21 days in a conditioned suppression paradigm that was terminated 2 weeks before the present experiments.

At 130 days of age (experiment I) and 105 days of age (experiments II-IV), the rats were killed by decapitation. Wet weights of thymus, adrenals, thyroids, and testicles were measured on a Sartorius balance. Any gross morphological changes were noted. Blood was drawn from the collected pool into the blood diluting pipette

for circulating eosinophil counts. The sample was treated as outlined by Miale (1967), while the counting procedure outlined by Wintrobe (1956) was followed using the Fuchs-Rosenthal counting chamber. A .5 ml blood sample was also taken for blood sugar analysis. A 1:10 protein free filtrate was prepared by mixing the .5 ml blood sample with .5 ml of 10% sodium tungstate, 3.5 ml distilled water, and .5 ml of 2/3N H_2SO_4 . After shaking well in a stoppered test tube, the solution was left standing for 10 min and filtered through No. 1 Whatman filter paper. The determination of the blood glucose level was then performed according to the Folin-Wu method (1920).

Results

The average relative thyroid weights and relative testicle weights for RMF-exposed, control, and sham-field rats are presented in Table 1. In experiment I, II, and III, the thyroid tissue of the RMF-exposed males averaged 738.0, 588.3, and 281.9 $\mu g/g$ (micrograms of thyroid tissue per gram of body weight) whereas that of the control males averaged 142.0, 201.8, and 248.3 $\mu g/g$. The differences between the 2 groups were found by *t*-test and Mann-Whitney *U* to be significant beyond the .05 level only in experiments I and II. In experiment IV, the group that had been exposed to the sham field averaged 245.0 $\mu g/g$ while the group exposed to the usual control conditions averaged 243.0 $\mu g/g$. This difference was not significant ($p > .05$). The thyroid of the RMF-exposed females in the three experiments averaged 581.3, 736.8, and 315.5 $\mu g/g$ while the control females averaged 184.8, 308.3, and 152.3 $\mu g/g$. The differences were significant in experiments II and III ($p < .05$). The average relative thyroid weights for the 3 control groups of either sex in experiments II-IV ($F = 1.10$) were not significantly different. The control groups from experiment I were not included in the analysis since they were 25 days older than the control groups in experiments II-IV.

The average relative testicle weights for the RMF-exposed males were 9.98, 11.72, and 12.17 mg/g whereas the controls averaged 8.39, 10.74, and 11.30 mg/g. These differences were not statistically significant at the .05 level ($.10 > p > .05$). However by combining the three experiments, the RMF-exposed groups averaged significantly heavier relative testicle weights than controls ($p < .01$). The average relative testicle weight of the rats exposed to the sham-RFM was 10.22 mg/g whereas the rats exposed to the usual control conditions averaged 10.39 mg/g. This difference was not significant. The percentage increase of thyroid and testicle weights of RMF-exposed rats with respect to control rats is presented in Table 2.

The differences in body weights taken immediately before decapitation for RMF-exposed males ($\bar{X} = 298.6 \pm 26.1$ g) and females ($\bar{X} = 226.8 \pm 16.1$ g) did not differ significantly from control males (317.4 ± 30.8 g) and females (234.8 ± 15.0 g). No significant differences were found between groups for eosinophil counts, adrenal weights, blood sugar, or thymus weights, although the RMF-exposed females had

TABLE 1. Relative Thyroid ($\mu\text{g/g}$ body weight) and Testicle Weight (mg/g) in Rats Exposed to RMF, Control, and Sham RMF Conditions.

		MALES				FEMALES	
		Rel Thyroid Weight		Rel Testicle Weight		Rel Thyroid Weight	
		RMF	Control	RMF	Control	RMF	Control
Exp I	$\bar{X}$	738.0	142.0	9.98	8.39	581.3	184.8
	SD	247.0	42.8	.16	1.98	371.9	53.0
	N	4	3	4	3	4	4
	t	4.00***		1.65		2.11	
	U	0.00**		0.00**		0.00**	
Exp II	$\bar{X}$	588.3	201.8	11.72	10.74	736.8	308.3
	SD	266.7	58.0	.75	.91	265.8	89.0
	N	8	4	8	4	4	3
	t	2.80**		2.02*		2.63**	
	U	4.00**		6.00*		0.00*	
Exp III	$\bar{X}$	281.9	248.3	12.17	11.30	315.5	152.3
	SD	61.9	33.9	.64	.81	48.9	71.2
	N	8	4	8	4	4	3
	t	<1		2.053*		3.63**	
	U	12.00		5.00*		0.00*	
		Sham-RMF	Control	Sham-RMF	Control		
Exp IV	$\bar{X}$	245.0	243.0	10.22	10.39		
	SD	126.0	39.1	.09	.02		
	N	4	4	4	4		
Exp IV	t	<1					

* $p < .10$ ** $p < .05$ *** $p < .01$

consistently lighter average relative thymus weights in the 3 experiments. Correlations between blood sugar and relative thyroid weights for RMF-exposed males (-.338) and females (-.618) were significantly different ($p < .01$) from control males (+.496) and female (+.582) correlations.

Discussion

Adult rats that have been exposed during different periods of their prenatal development to a .5 Hz, 0.5-3 or 3-15 gauss RMF show a marked average increase in

TABLE 2. *Percentage Increase of Thyroid and Testicle Weights of RMF Exposed Rats Compared to Controls in Experiments I, II, and III, and Sham RMF Compared to Controls in Experiment IV, as a Function of Duration of Exposure and Field Intensity.*

Experiment	Tissue		Exposure Duration (in days)	Intensity (in gauss)	
	Thyroid				Testicle
	Male	Female			
I	420%	214%	19%	1-22 [*]	3-15
II	191%	139%	9%	19-22	3-15
III	14%	108%	8%	1-22	0.5-3
IV	1%	-	-2%	1-22	sham

*Day 22 = day of birth.

relative thyroid weight and testicle weight. However, the variance of the RMF-exposed rats' thyroid weights is about 2 to 3 times higher than controls. It is interesting that the ambulatory behavior of other RMF-exposed rats, although on the average less than controls, also shows about twice as much variance (Persinger, 1971). These data suggest that other variables, in addition to the RMF, are associated with the thyroid weight changes. A possibility is that varying ambient ELF fields, which were not shielded out in this study, contributed to the effect of the RMF.

The reliability of the observed effect is indicated by the replications over experiments, the tendency for a "dose" relation, and the absence of significant thyroid and testicle weight differences between sham-RMF and control animals or between control animals of the same age in the various experiments. In all 3 experiments, both male and female RMF-exposed rats averaged heavier thyroids than controls. The greatest difference in thyroid weights was between the rats exposed prenatally to the 3-15 gauss field during their entire prenatal development, whereas the least difference occurred between rats exposed to the .5-3 gauss field during the same period. The rats exposed to the sham-field did not differ from controls but their variance was greater. Although the variance might be attributed to some other artifact of the apparatus, some residual magnetism (< 0.5 gauss) remained on the output shafts even with the magnets removed. The reliability of the results is given additional support since both RMF-exposed males and females with different behavioral histories had consistently heavier average thyroid weights than controls.

The data of Piccardi (1962) concerning VLF effects on colloids could implicate the thyroid as a major tissue structure affected. Thyroid follicles do contain relatively large concentrations of colloid in water solutions. If these macromolecules ($gmw > 700,000$) have great intermolecular forces, they might behave as liquid crystals (Ludwig, 1968). The Debye-absorption frequencies in liquid crystals are, owing to the great intermolecular forces, in the same range as VLF signals (Meier &

Saupe, 1966). Hence, if the thyroid colloid satisfied these physicochemical properties, at least VLF absorption would be possible. Stewart (1959, 1961, 1969) reports that some endocrine tissues do contain liquid crystalline material. Whether the change in thyroid and testicle weight is related to similar ELF absorption, decrements in respiration rate (Lotmar, et al., 1969, a,b), and disturbances of blood clotting (Piccardi, 1962) requires further investigation.

Although increased thyroid weight is not indicative of thyroid activity, some data do suggest that RMF-exposed rats are more "hypothyroid" than controls. The Maudsley reactive strain, which was bred over generations for high defecation (Broadhurst, 1962) also shows less activity in the open field, greater conditioned suppression (Singh, 1959), larger thyroids, and in addition, less thyroxine and protein-bound iodine in serum with slower rates of ^{131}I uptake (Feuer & Broadhurst, 1962, a,b,c). Such relative increases in thyroid weights for "emotional" (high defecation) rats compared to "nonemotional" (low defecation) rats have been reported by other experimenters as well (Yeakel & Rhoades, 1951). Ossenkopp (1972) noted that rats exposed during their entire prenatal development to a .5 Hz, 3-12 gauss RMF (different apparatus than the one used in this study) showed a delay in eye opening and teeth eruption. The latter two changes are characteristic of rats thyroidectomized at birth (Eayrs & Lishman, 1955). Thyroidectomy also influences the developing nervous system (Hamburgh, 1969; Eayrs, 1959) and is associated with changes in brain chemistry and morphology. Geel and Timiras (1967) have shown that neonatal thyroidectomy decreased AChE and ChE content in the cerebral cortex. Meisame, Valcana, and Timiras (1970) concluded that the brain of the hypothyroid rat was more "excitable", at least to shock induced seizures. If indeed prenatal ELF exposure did simulate a "functional thyroidectomy", then an increased reactivity to novel or aversive stimuli would be probable. The behavior of prenatally ELF-RMF-exposed rats has been described in this manner (Persinger & Pear, 1972). One possible way to increase the thyroid effect might be to expose neonates to the ELF-RMF during the time (postnatal Days 1-5) when surgical thyroidectomy produces the greatest consequent effects (Eayrs, 1968).

The present study suggests that prenatal exposure to natural ELF signals might produce similar changes in adult tissue. Admittedly, the magnetic component of the ELF field used was much higher than that which occurs in nature. However, Ludwig (1968) has conjectured that amplitude should be of minor importance, frequency being the more critical variable. Lotmar et al., (1969 a, b) noted that the magnetic component alone did not produce the changes in respiration rate in mouse liver tissue, rather it appeared that the electrical component of the changing magnetic field was also critical. In the present study the electrical component was 10^{-5} V/m, well within the range of natural intensities.

The results of this study support 2 statements: (1) that prenatal exposure to ELF fields is associated with measureable changes in physiology, and (2) that the thyroid seems to be one of the major tissue complexes affected. Further experimentation must

be done in order to understand the biochemical processes associated with changes in thyroid weight as well as testicle and possibly thymus weight and activity. Such investigations are considered important since, as Graf et al. (1967) have pointed out, life evolved on this planet in pulsating electromagnetic fields. Their day-to-day variations have been reported to be associated with marked changes in living organisms. The alteration of these fields, as man probes deeper and more often into space, may produce undesirable behavioral and physiological changes (Becker, 1963).

Notes

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